EXHIBIT A

2/15/00

Clean DNA first.

Run a gel

there are just a

little DNA in expect size

so improve Digest

Digest

DNA 40 ul buffer 10 ul

H2O 42.5 ul EcoR I 8.5 ul } overnight 37°C

GENOSYS
HAPDAMP2
5'-TGTGCCGTTTGGTACCTATA

GENOSYS
HAPDAMP2
5'-TGTGCCGTTTGGTACCTATA

→ primer

GENOSYS
HAPDAMP2
5'-TGTGCCGTTTGGTACCTATA

← New

2/17/00

Digest — to ~~check~~ differential digest procedure for dam gene & detection

11039 or D753 DNA 1 ul

10 x buffer 1 ul

H2O 7.5 ul

enzyme 0.5 ul

↓

3 digest Sau 3A I Dpn I Mbo I

↓ ↓

both unmethylated gene do-methylated gene

Digest 37°C 4 hr.

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Inverse PCR procedure

Use 100 ul EcoRI digest set up 5 digestions

	1	2	5
1039 EcoRI cut DNA			
10× buffer	5	5	5
T4 ligase (WTB)	0.5	0.5	0.5
H2O	33.5	33.5	33.5

50 ul

16°C overnight heat inactivate

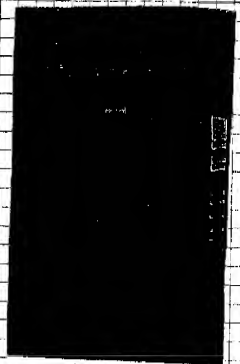
for PCR

2-2-100

Run a gel to detect differential digest procedure for dam gene

Ladder ① 1039 Sau3AI ② 1039 MboI ③ 1039 DpnI

④ DI53 ⑤ DI53 ⑥ DI53



⑥ ⑤ ④ ③ ② ①

There once some is dam gene in DI53.

Sau3AI → digest GATC both methyled and unm-

MboI → digest GATC only un---

DpnI → digest GATC only methylated.

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